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University of Dundee
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United Kingdom

Patents ADP number *(if you know it)* 7822760001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — Cytochrome P450

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Cytochrome P450

This invention relates to a retinoic acid metabolizing cytochrome P450 (CYP450) and to sequence specific primers and assays for the analysis of cytochrome P450 2S1 (CYP2S1) messenger RNA expression.

The methods may be used for the identification of modulators of CYP2S1 expression which are useful in the treatment of skin disorders such as psoriasis.

The invention also includes a method of identifying a modulator of CYP2S1 activity comprising: [a] incubating CYP2S1 or a cell expressing CYP2S1 with a test compound under conditions that modulate CYP2S1 expression or activity; [b] detecting the activity or expression, of CYP2S1 in the presence of said test compound, a decrease in said activity or expression being indicative that the test compound is an inhibitor of CYP2S1 expression or activity, while an increase in said expression or activity is indicative that the test compound is an activator of CYP2S1 expression or activity. The invention further provides a method of treating or preventing a disease associated with CYP2S1 expression comprising administering an effective amount of an agent that activates, simulates or inhibits CYP2S1 expression, as the situation requires.

A preferred embodiment of the invention is a method of modulating CYP2S1 expression where such modulation either increases or decreases metabolism of all trans retinoic acids in the treatment of skin disorders in particular psoriasis.

A still further aspect of the invention are methods of modulating CYP2S1 activity in any cell type or tissue, including tumours resulting in changes in retinoic acid or its

pharmacophors. Modulation of CYP2S1 can be achieved by use of inhibitors or activators of gene transcription or inhibitors or activators of the protein. Tests would also include transactivation assays using the CYP2S1 promoter. It may also be desirable to express CYP2S1 in skin for gene therapy.

Background Inter-individual differences in response to topical drug treatment and photo(chemo)therapy are a significant clinical problem in the treatment of common skin diseases such as psoriasis. Individuality in hepatic drug metabolising enzyme expression is an important determinant of systemic drug handling; similar variation in cutaneous gene expression may therefore contribute to individuality in response to topical therapies.

Methods We used quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) to demonstrate the expression of a novel cytochrome P450, CYP2S1, in human skin in healthy subjects and patients with psoriasis. We also investigated CYP2S1 regulation by ultraviolet radiation (UVR), psoralen-Ultraviolet photochemotherapy (PUVA) and topical drugs used to treat psoriasis.

Findings We have shown that the novel human P450, CYP2S1, is expressed in human skin and demonstrated marked individuality in constitutive CYP2S1 expression and induction following UVR or topical drug treatment. Cutaneous CYP2S1 expression was induced by UVR, PUVA, coal tar and *all-trans* retinoic acid (atRA) and was significantly elevated in lesional psoriatic skin, implying that topical drugs may be differentially metabolised in psoriatic plaque compared with non-lesional skin. We demonstrated that atRA is metabolised by CYP2S1, the cutaneous

expression of which is significantly higher than CYP26, previously described as the specific cutaneous P450 retinoic acid metabolising enzyme.

Interpretation These data increase our understanding of the interaction between therapeutic agents and the skin and suggest a functional role for CYP2S1 in the metabolism of topical drugs, and in mediating the response to photo(chemo)therapy in psoriasis patients.

Introduction

Human skin is the largest organ of the body. In addition to its role as a physical barrier, there is increasing evidence that skin is metabolically active. For example, it has recently been reported that human skin expresses a diverse and functional CYP450 monooxygenase system[1-4]. CYP450s have been extensively studied in other organs, particularly liver, where they are essential catalysts of drug and xenobiotic metabolism. There is marked inter-individual variation in the expression and activity of many hepatic CYP450s, resulting in inter-individual differences in response to drug therapies and in the incidence of adverse drug reactions[5].

Skin disease is common, with one third of the population affected at any one time[6]. Psoriasis affects approximately 2% of the population and is a major cause of morbidity and a significant therapeutic challenge. Inter-individual differences in response to topical drug treatment and ultraviolet radiation (UVR) in the treatment of common skin disorders, such as psoriasis, are unpredictable. While the majority of patients tolerate drug therapy, a proportion develop adverse effects or is "treatment-resistant". Similarly, it is not possible to predict which patients will respond rapidly and well to ultraviolet B (UVB) phototherapy or psoralen-ultraviolet A (PUVA)

photo(chemo)therapy or those who will be less responsive. An understanding of the factors which determine how individual patients respond to drug or UVR therapies may enable the outcome of treatment to be more predictable, thus reducing side effects and facilitating the selection of optimal treatment regimens on an individual basis.

One factor which may contribute to variation in response to treatment is inter-individual differences in the cutaneous expression of drug metabolising enzymes and cytoprotective genes. Human skin is the first line of defence against environmental insult and it seems logical that it would be a rich source of cytoprotective genes, thought to have evolved as an adaptive response to environmental challenge[7]. Relatively little is known about the expression and regulation of these genes in human skin although they have been extensively studied in liver and in rodent and cell culture models.

CYP450s are a multigene family of Phase I monooxygenases which, together with their redox partner NADPH cytochrome P450 reductase (CPR), catalyse the oxidative metabolism of the vast majority of drugs and xenobiotics to which we are exposed[8]. CYP450s in subfamilies CYP1 to CYP4 are responsible for the majority of foreign compound metabolism, have unique but overlapping substrate specificities and, in general, are regulated by substrate-induced activation of gene transcription[9]. CYP450 expression is highest in the liver, but many CYP450s are also expressed at significant levels in extra-hepatic tissues[10]. CYP450 expression was first described in human skin more than 20 years ago[11] and was shown to be inducible following the topical application of crude coal tar, a source of polycyclic aromatic hydrocarbons (PAHs),

known to be potent inducers of CYP450 gene transcription[12]. Constitutive expression of CYP1A1 and the closely related isozyme CYP1B1 in human keratinocytes was recently confirmed by RT-PCR in a series of experiments which also identified mRNAs encoding CYP2B6, CYP2E1 and CYP3A5[1]. In a complementary study, mRNAs encoding CYP450s CYP2A6, CYP2B6 and CYP3A4 were identified in primary human keratinocytes using RNase protection analysis[2]. Interestingly, recent data suggests that Ultaviolet B (UVB) irradiation can induce the cutaneous expression of CYP1A1, CYP1B1[3] and CYP4A11[4]. An increased understanding of cutaneous gene expression and regulation by topical chemicals and photo(chemo)therapy and the ability to determine individual patient phenotypes may lead to a more rational approach to the treatment of common skin diseases.

A novel CYP450, CYP2S1, has been identified from human genome databases. This enzyme is primarily expressed in extra-hepatic tissues including the trachea, lung and small intestine[13]. CYP2S1 has been localised to chromosome 19q13·2, in a cluster with other CYP2 family CYP450s which are known to be involved in drug and xenobiotic metabolism[14]. Using quantitative real-time RT-PCR, we have investigated CYP2S1 expression in human skin from healthy volunteers and psoriasis patients and demonstrated its regulation by UVR and PUVA, and by specific topical drugs used in the treatment of psoriasis. We have additionally investigated whether there are inter-individual differences in constitutive CYP2S1 expression and inducibility by UVR and drug treatment.

Methods

Study participants

Patients with chronic plaque psoriasis, attending the Photobiology Unit, Ninewells Hospital, Dundee in order to commence UVB phototherapy (n=26) or oral 8-methoxy psoralen photo(chemo)therapy (PUVA) (n=3), were invited to participate in the study. Healthy volunteers (n=27), recruited by local advertising, were invited to participate in the topical chemicals study. All study participants were Caucasian and resident in Tayside at the time of recruitment. Informed consent was obtained from all study participants and the study was approved by the Tayside Committee on Medical Research Ethics.

Irradiation was performed on photoprotected buttock sites of psoriasis patients using either a solar simulator UVR source (150 W xenon arc lamp, 290 – 400 nm, 770 mW/cm^2, 3·9-48 J/cm^2,) (n=26), or a UVA dose series (Waldmann F15W/T8, 320 – 400 nm, 5·7 mW/cm^2, 0·5 – 7·9 J/cm^2) 2 h after ingestion of 8-methoxypsoralen (0·6 mg/kg, Meladinine®, Galderma, UK) (n=3). At 24 h after solar simulator irradiation or 72 h after PUVA exposure, the minimal dose of radiation required to induce a just perceptible erythema (the minimal erythema dose (MED) or minimal phototoxic dose for PUVA (MPD)) was determined and biopsies performed. Full thickness (4mm diameter) punch biopsies were taken from (a) an irradiated site (1-4 x MED or 3-4 x MPD), (b) adjacent untreated psoriatic plaque and (c) adjacent control site.

To study the effects of topical chemicals, crude coal tar (12% (w/w) in petrolatum) or *all-trans* retinoic acid (atRA) (0·025% (w/w) Retin A® gel) and vehicle control were occluded on aluminium Finn chambers on photoprotected buttock sites of healthy volunteers for 96 hours. After removal of the chambers, full thickness punch biopsies

(4 mm diameter) were taken from chemically treated sites and adjacent control skin. All biopsy samples were immediately snap frozen in liquid nitrogen and stored at – 70°C prior to analysis.

RNA preparation and cDNA synthesis

Total RNA was extracted from 4mm diameter skin punch biopsies using Qiagen RNeasy spin columns, according to the manufacturer's instructions, with the addition of Proteinase K and DNase digestion steps. Total RNA from other tissues was prepared using the Promega SV Total RNA isolation system, according to the manufacturer's instructions. RNA concentration and purity was determined spectrophotometrically by measuring fluorescence at 260nM and 280nM. Total RNA (200ng) was reverse transcribed into cDNA in a total volume of 50µl using PE Applied Biosystems Taqman Reverse transcription reagents according to the manufacturer's instructions. 1µl, corresponding to approximately 4 ng of input RNA was used in subsequent Taqman analysis.

Taqman quantitative real-time PCR analysis

Sequence-specific primers and probes for Taqman quantitative PCR analysis of CYP2S1 mRNA expression were designed using PE Applied Biosystems Primer Express software, according to the manufacturer's protocol. PCR (1 x (50°C, 2 minutes, 95°C, 10 minutes), 40 x (92°C, 15 seconds, 60°C, 1 minute)) was performed in the presence of 0·6 x Taqman Universal PCR Master Mix (PE Applied Biosystems), 300nM forward primer (5'-CGA TGC CTT CCT GCT GAA G-3'), 300nM reverse primer (5'-GCA TGT TCT TGT TGG TGA ATT CTG 3') and 175

nM fluorescent probe (5'-FAM-TGG CAC AGG AGG AAC AAA ACC CAG G-3'). The assay was designed such that the probe spanned an intron/exon boundary to minimise the possibility of co-amplifying genomic DNA. Similarly, assays were designed for CPR (Forward primer 5' CCT GCA GGC CCG CTA CTA 3', Reverse primer 5' TTG GTC TCG TAC TCC ACA ACC A 3', Probe 5'-FAM- TCC TCC AAG GTC CAC CCC AAC TCT GT 3') and CYP26 (Forward primer 5' CCG TAT TTC CTG CGC TTC AT 3', Reverse primer 5' TTC CCC TTC TTT GGG GAA AC 3', Probe 5'-FAM- CAG GAA CTT CCT CCG CTG CAG TAC CAT 3'). Real-time PCR was performed on an ABI Prism 7700 Sequence Detector, where fluorescent output was directly proportional to input cDNA concentration. Input cDNA concentrations were normalised to 18S ribosomal RNA, using PE Applied Biosystems Ribosomal RNA control reagents. Oligonucleotide primers were synthesised by MWG Biotech and fluorescent Taqman probes by PE Applied Biosystems.

CYP2S1 promoter analysis

To date, the CYP2S1 cDNA sequence (NCBI Accession number NM_030622) is the only published CYP2S1 sequence information. In order to identify the CYP2S1 promoter, the CYP2S1 cDNA sequence was aligned with a genomic clone (NCBI Accession number NT_011139) containing a region of chromosome 19 encompassing the CYP2 CYP450 cluster[14]. A 10kB region immediately upstream of the CYP2S1 transcription start site representing the CYP2S1 gene promoter was analysed for the presence of specific regulatory elements using MatInspector v2·2 software[15].

atRA metabolism by heterologously expressed CYP2S1

The CYP2S1 cDNA, a generous gift from Professor Oliver Hankinson, Department of Pathology and Laboratory Medicine, Jonsson Comprehensive Cancer Centre and Molecular Biology Institute, UCLA, Los Angeles was constructed and co-expressed with CPR in *E. Coli* as previously described[16]. CYP2S1 metabolism of atRA was performed using HPLC analysis as previously described[17], using *E. coli* membranes co-expressing CYP2S1 and CPR and an NADPH regenerating system. Control reactions were performed in the absence of CYP2S1 and in the absence of the NADPH regenerating system. CYP2S1 metabolites were identified by comparison with elution times and UV profiles of atRA metabolites generated by human liver microsomes which had previously been identified using authentic standards.

Statistical analysis

Triplicate measurements of skin mRNA expression were made from each sample, and the mean value taken. After logarithmic transformation, mRNA expression values in control and "lesional" (irradiated, psoriatic plaque or chemically-treated) skin followed a Gaussian distribution, and the paired t-test was used for statistical comparisons of values in "lesional" versus control skin. The possibility of an association between UV dose (in MED-multiples) and relative induction was explored graphically and by Spearman rank correlation, with a confidence interval for the correlation coefficient (r_s) derived by 'bootstrapping" with 2000 replications. Stata (Intercooled Stata for Windows version 7·0, Stata Corp, Tx, 2002) software was used.

Results

1. Constitutive CYP2S1 expression in human skin

We investigated whether CYP2S1 was expressed in human skin and compared the level of cutaneous expression with other extra-hepatic tissues, including small intestine and kidney, which had previously been reported to express CYP2S1[13] (Figure 1). As expected, we found that extra-hepatic CYP2S1 expression was higher than in human liver. In addition to the small intestine and kidney, CYP2S1 was expressed in breast, placenta, large intestine and human skin. In this initial experiment, CYP2S1 mRNA expression appeared to be induced by UVR exposure and to be elevated in lesional psoriatic skin.

2. Induction of CYP2S1 expression by UVR

To investigate the effects of UVR exposure on cutaneous CYP2S1 expression, we compared CYP2S1 mRNA levels in non-lesional skin of patients with psoriasis, exposed to a solar simulator at 1-4 x MED UVR with non-lesional untreated skin (n=26) (Figure 2A). CYP2S1 was induced by UVR, with the geometric mean expression 1·78 (95% CI 1·39 to 2·27) times higher in irradiated compared with control skin (p=0·0001). Marked inter-individual variation in both constitutive CYP2S1 expression (up to 5-fold variation) and UVR-inducibility (up to 2-fold variation) was seen. Doses of UVR ranged from 1 x MED to 4 x MED UVR (2-3 x MED in 20/26 patients, Figure 2A). There was no detectable correlation between UVR dose and induction of CYP2S1 in irradiated skin (r_s=0·30 (95% CI 0·09 to 0·60), p=0·13). Interestingly, we also observed marked induction of CYP2S1 mRNA

expression in a smaller number of patients (n=3), 72 hours after exposure to 3-4 x MPD psoralen-UVA (PUVA) irradiation (Figure 2A).

3. Expression of CYP2S1 in lesional psoriatic skin

CYP2S1 mRNA expression was compared in biopsies taken from untreated lesional psoriatic skin and adjacent non-lesional control skin (n=29) (Figure 2B). CYP2S1 was consistently higher in psoriatic skin, with the geometric mean expression 3·38 (95% CI 2·64 to 4·34) times higher in psoriatic compared with control skin ($p<0·0001$). Again, there were significant inter-individual differences in CYP2S1 inducibility between patients (range 0·58 to 15·53). Interestingly, we also saw marked induction of CPR mRNA levels in psoriatic skin (median induction 3·09, 95% CI 2·30 to 5·11, range 1·23-16·60).

4. CYP2S1 promoter analysis

In order to investigate possible mechanisms of CYP2S1 regulation, we examined sequences in the CYP2S1 gene promoter to identify regulatory elements which may influence CYP2S1 expression and inducibility. No CYP2S1 promoter sequence has been published to date, but the CYP2S1 gene has been mapped to chromosome 19q13·2[13]. We therefore identified the CYP2S1 gene promoter by mapping the CYP2S1 cDNA onto a genomic clone (NCBI Accession number NT_011139) containing the CYP450 cluster on chromosome 19[14]. Recent evidence suggests that transcriptional regulation of CYP450 genes can involve distal regulatory sequences, with a potent enhancer sequence as far as 8kb distal to the transcription start[18]. In light

of these findings, we analysed a 10kb fragment of the CYP2S1 promoter, including the sequence immediately preceding and including the transcription start site.

A number of putative transcription factor binding sites were identified, including two sites with complete homology to the AP-1 consensus sequence (5'-TGA(G/C)T(C/A)A-3') and multiple "AP-1 like" sites differing from the AP-1 consensus sequence by a single nucleotide. Interestingly, we also identified several transcription factor binding sites which are known to regulate hepatic CYP450 expression and which may therefore influence cutaneous response to drug and xenobiotic challenge. These include the xenobiotic response element (XRE) which mediates CYP450 induction in response to polycyclic aromatic hydrocarbons[19], and the retinoic acid receptor response element (RARE)[20]. The CYP2S1 promoter contains 2 XRE sequences (5'-GCGTGCAC-3') identical to the XRE sequences in the major PAH-inducible CYP450s CYP1A1, CYP1A2 and CYP1B1. Retinoic acid receptors (RARs) activate gene transcription in the presence of *all-trans* retinoic acid (atRA) by binding to retinoic acid promoter response elements (RAREs) as heterodimers with retinoid X receptors (RXRs)[21]. RAREs consist of two half-sites with the consensus sequence (5'-AGGTCA-3'), occupied by RAR and RXR respectively, arranged as direct repeats spaced by a single (DR1) or five (DR5) nucleotides[22]. Multiple RAR consensus half-site sequences are present in the CYP2S1 promoter.

5. Induction of CYP2S1 expression by topical chemicals

In light of the above analysis, we investigated whether CYP2S1 expression was regulated following the topical application of drugs used routinely in the treatment of psoriasis and other skin diseases. Specifically, we investigated the effects of topically applied crude coal tar (an abundant source of polycyclic aromatic hydrocarbons) and *all-trans* retinoic acid. CYP2S1 mRNA levels were determined following topical drug application and compared with a vehicle control site. We had previously confirmed that, as expected from previous literature[23], coal tar treatment consistently induced, and atRA suppressed CYP1A1 mRNA expression in our volunteer panel, suggesting that both chemicals had been sufficiently absorbed and had been applied at doses which modulated gene transcription (data not shown). Crude coal tar (Figure 3A) and atRA (Figure 3B) were found to induce CYP2S1 mRNA expression, but only in a subset of individuals, with 6/13 coal tar-treated individuals and 6/14 atRA - treated individuals showing marked CYP2S1 induction.

6. CYP2S1 makes a significant contribution to cutaneous retinoic acid metabolism

CYP2S1 appears to be regulated by atRA in human skin. The observation that CYP450 inducers are also substrates for these enzymes lead us to investigate whether atRA was a CYP2S1 substrate and whether CYP2S1 made a significant contribution to the regulation of cutaneous atRA levels. CYP2S1 was expressed in *E. Coli* together with CPR, using an approach described previously for other CYP450 enzymes[16] and the atRA metabolites produced by heterologously expressed CYP2S1 characterised by HPLC analysis[17]. CYP2S1 produced two distinct atRA metabolites which, by comparison with the metabolite profile produced by human liver

microsomes, were identified as 4-OH-RA and 5,6-epoxy RA (Figure 4A). In contrast, CYP2S1 did not appear to produce the 4-oxo RA metabolite, which was produced by human liver microsomes. (Figure 4B). The identity of this additional metabolite was confirmed using an authentic standard for 4-oxo RA (data not shown).

To determine whether CYP2S1 made a significant contribution to atRA metabolism in human skin, cutaneous CYP2S1 expression was compared to CYP26, a CYP450 which was recently identified as a substrate-inducible and highly specific cutaneous retinoic acid metabolising enzyme[24] (Figure 5). Cutaneous CYP26 expression was extremely low compared to CYP2S1, even following induction with atRA.

Discussion

The difficulty in predicting clinical response or risk of toxicity from topical drug treatment or photo(chemo)therapy is a major clinical problem in the management of common skin diseases, such as psoriasis. The metabolic role of skin is increasingly recognised and inter-individual differences in the cutaneous expression and regulation of drug metabolising enzymes and cytoprotective genes may explain variation in clinical response. Cytochrome CYP450s play a central role in hepatic drug and xenobiotic metabolism, show marked inter-individual differences in catalytic activity and are therefore attractive candidates which may contribute to individuality in cutaneous therapeutic responses.

A number of CYP450 isozymes make a significant contribution to drug metabolism in extra-hepatic tissues[10] and there is mounting evidence that skin, the largest organ of the body, is a metabolically active organ with a diverse and functional CYP450

monoxygenase system[1-4,13]. We have demonstrated for the first time that a newly identified CYP450, CYP2S1, is expressed in human skin and is inducible by UVR (290-400nm) and PUVA (Figure 2A). Interestingly, analysis of the CYP2S1 promoter revealed the presence of multiple copies of the AP-1 transcription factor binding site, which is known to mediate induction of cutaneous gene transcription in response to a variety of extracellular stimuli including UVR[25]. AP1 binds c-fos/c-jun heterodimers, both of which have been shown to be expressed in the skin[26] and, through increased transcription of c-fos and c-jun, is known to mediate UVR induction of the cutaneous expression of genes including the matrix metalloproteinases interstitial collagenase (MMP-1) and stromelysin (MMP-3)[27].

Interestingly, the expression of CYP2S1 was significantly elevated in psoriatic plaque. Psoriasis is a chronic hyperproliferative and inflammatory disorder which results in the production of reactive oxygen species (ROS) and proinflammatory cytokines such as interleukin 1 (IL-1) and tumour necrosis factor α (TNFα)[28]. The marked induction of CYP450 expression in psoriatic skin is of particular interest as the majority of previous literature suggests that CYP450 expression is suppressed in response to inflammatory mediators[29]. A number of transcription factors including AP-1 and NF-κB have been shown to mediate the effects of inflammatory mediators and ROS on gene transcription[25,30]. In addition to the AP-1 consensus binding sites described previously, the CYP2S1 gene promoter contains several NF-κB consensus binding sites. We found a highly significant correlation between induction of CYP2S1 mRNA expression by UVR and in psoriatic plaque in individual patients ($p=0{\cdot}003$),

suggesting that a common mechanism may mediate the effects of ROS and inflammatory mediators on CYP2S1 gene transcription.

CPR was also significantly induced in psoriatic plaque which, in conjunction with the marked increase in CYP2S1 expression, implies a global up-regulation of CYP2S1-mediated metabolism and clearance in lesional psoriatic skin compared with surrounding non-lesional skin. Consistent with this model, it is a common observation in clinical practice that peri-lesional or non-lesional skin of psoriasis patients is more susceptible than lesional psoriatic skin to irritancy following treatment with coal tar or dithranol and is more likely to develop phototherapy-induced erythema.

Significant induction of CYP450 expression in lesional psoriatic skin has implications for the efficacy and tolerability of topical drug therapy, used routinely in the treatment of psoriasis. Based on the relatively high expression of CYP2S1 in skin and its chromosomal localisation in the centre of a cluster of xenobiotic metabolising CYP450s, we would predict that it may play an important role in cutaneous drug metabolism. Topical retinoids are used routinely in the treatment of psoriasis and their metabolism is complex, with the CYP450-catalysed metabolism of at-RA resulting in the formation of many metabolites including 4-OH-RA, 4-oxo-RA, 18-OH-RA and 2,6-epoxy-RA[16]. We have shown that cutaneous CYP2S1 expression is significantly higher than CYP26, previously identified as a highly specific and substrate-inducible retinoic acid metabolising enzyme[24]. The role of CYP26 in retinoic acid metabolism is also described in WO02095034. We have additionally investigated the cutaneous expression and regulation by ATRA of additional

CYP450s, including the CYP3A family genes and CYP2C8, which have been shown to contribute to hepatic ATRA metabolism[16]. Of these genes, only CYP3A5 was constitutively expressed in human skin and was not inducible by ATRA. In light of the fundamental role played by retinoids in regulating gene expression, in development, and in the maintenance of epithelial tissues, we propose that CYP2S1 is an important determinant of cutaneous retinoid homeostasis.

We have also shown that topical coal tar induces cutaneous CYP2S1 expression and identified multiple XRE consensus elements in the CYP2S1 promoter, leading us to predict that PAHs and other CYP1 family substrates would also be metabolised by CYP2S1. It has also been identified that CYP2S1 is a novel dioxin-inducible CYP450[31].

Interestingly, we saw significant inter-individual differences in CYP2S1 inducibility in response to atRA and coal tar treatment, with a significant proportion of treated individuals showing no induction of CYP2S1 mRNA expression. This result is intriguing as we have previously shown consistent induction of other PAH-inducible CYP450s including CYP1A1 and CYP1B1 by coal tar in the same volunteer panel, suggesting that all the components of the Ah receptor/XRE transcriptional complex are present and functional in all subjects. It appears that inter-individual differences in CYP2S1 inducibility could be explained by genetic polymorphism. The expression of many CYP450s, including CYP1A1, CYP2C19 and CYP2D6, is subject to genetic polymorphism, resulting in a diverse population distribution of alleles, often with significantly different catalytic activities[32]. CYP2S1 is an obvious target for

polymorphism screening and we are currently investigating the extent to which genetically determined variation in CYP2S1 expression or inducibility may rationalise inter-individual differences in response to topical drug treatment.

CYP2S1 regulation by UVR, PUVA and by topical drugs used to treat psoriasis raises the possibility that the combination of photo(chemo)therapy and topical drug treatment may have additive or synergistic effects on CYP2S1 expression and inducibility. In support of this hypothesis, there is evidence that the combination of crude coal tar and UVA causes a greater suppression of epidermal DNA synthesis than either single treatment[33] and that enhanced clearance of psoriasis can be achieved by combination therapy with topical coal tar and UVR[34].

We have shown that CYP2S1 is expressed in human skin and is induced by UVR, PUVA, selected topical therapeutic chemicals and is elevated in lesional psoriatic skin. We have also shown marked inter-individual differences in response to chemical and UVR exposure. These findings suggest that CYP2S1 may be an important determinant of individuality in cutaneous response to topical drug treatment and photo(chemo)therapy and increase our understanding of the interaction between the skin as a metabolically active organ and the environment.

Figure Legends

Figure 1 Constitutive CYP2S1 mRNA expression in various human tissues.

Quantitative real-time RT-PCR was used to compare CYP2S1 mRNA expression in a panel of human tissues including skin, breast, kidney, liver, placenta and regions of

the gastrointestinal tract. CYP2S1 mRNA expression in UVR-treated non-lesional skin, in psoriatic plaque and in adjacent untreated control skin within the same subject is shown. Each of the other non-cutaneous tissue samples was obtained from a different individual and was analysed in triplicate. CYP2S1 expression was normalised to 18S ribosomal RNA to ensure equality of loading. Results are presented as the mean of triplicate determinations, ± standard error.

Figure 2 Fold change in CYP2S1 mRNA expression in patients exposed to UVR or in psoriatic plaque

CYP2S1 mRNA expression in UVR irradiated non-lesional skin (<2xMED, light green; 2-3xMED, blue; 4xMED, turquoise bars) compared with adjacent untreated non-lesional control skin in the same patients with psoriasis. Expression in PUVA-treated skin is also shown (red bars) (Figure 2A). CYP2S1 mRNA expression in psoriatic plaque compared with adjacent untreated non-lesional control skin in the same patients (Figure 2B). Each data point represents fold change in CYP2S1 expression in one patient.

Figure 3 Fold change in CYP2S1 mRNA expression by topical chemicals

CYP2S1 mRNA expression in healthy volunteers treated on photoprotected buttock sites with either crude coal tar (12% (w/w) in petrolatum) (n=13) (Figure 3A) or *all trans* retinoic acid (0·025% (w/w) RetinA® gel) (n=14) (Figure 3B). Data represent gene expression in chemically-treated skin compared to adjacent untreated control skin expression in the same individuals. Each data point represents fold change in CYP2S1 expression in one subject.

Figure 4 Metabolism of atRA by CYP2S1

E. Coli membranes co-expressing CYP2S1 and CPR were incubated with atRA as previously described[17]. CYP2S1-derived atRA metabolites (Figure 4A) were separated by HPLC and identified by comparison with the elution profile of human liver microsomes (Figure 4B). Control incubations were performed in the absence of the NADPH regenerating system (Figure 4C).

Figure 5 Expression of CYP2S1 relative to CYP26 in human skin

Quantitative real-time RT-PCR was used to compare CYP2S1 and CYP26 mRNA expression in human skin before and after treatment with atRA. Target gene expression was normalised to 18S ribosomal RNA to ensure equality of loading. All samples were analysed in triplicate.

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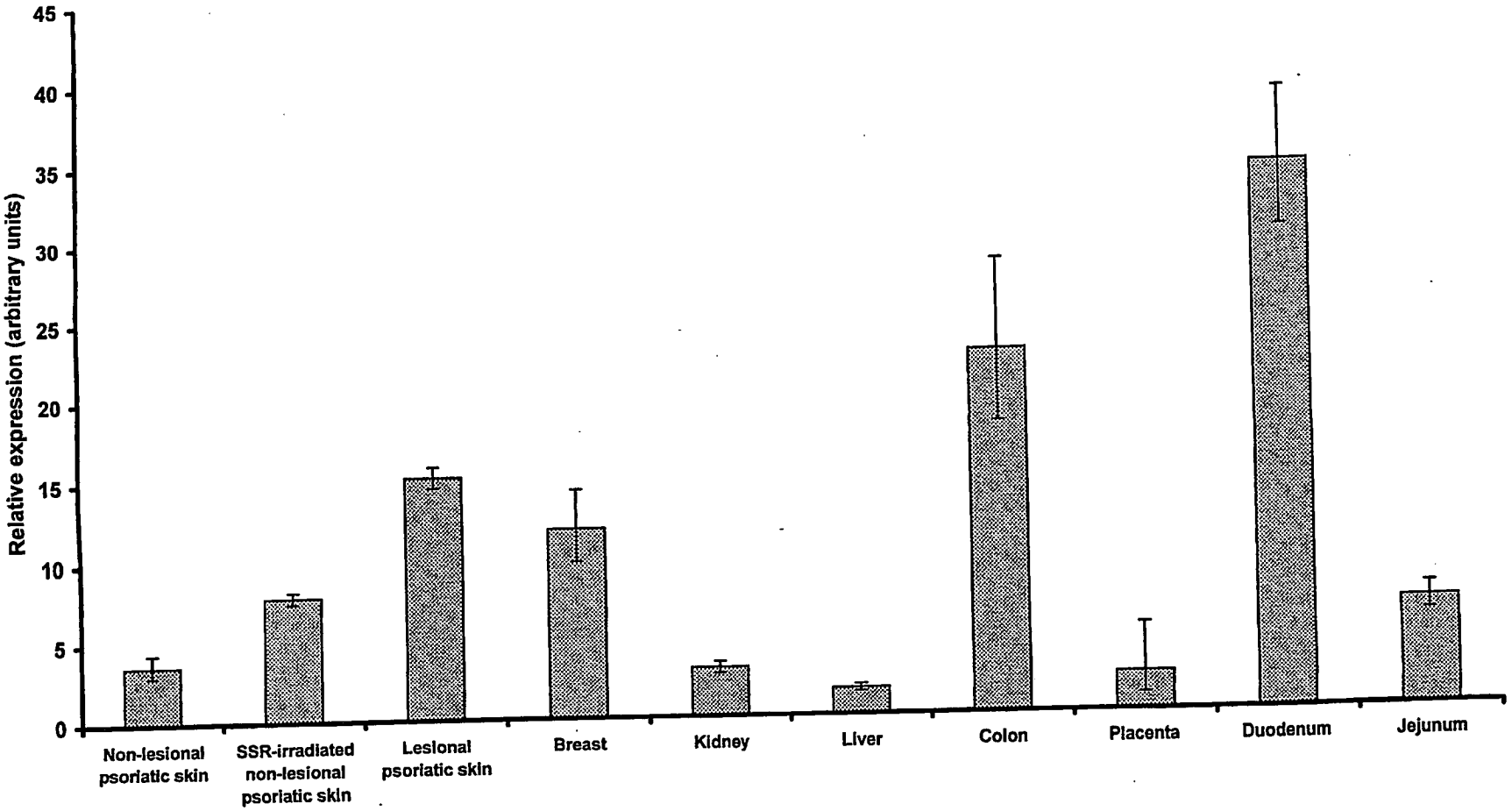
Figure 1


Relative expression (arbitrary units)
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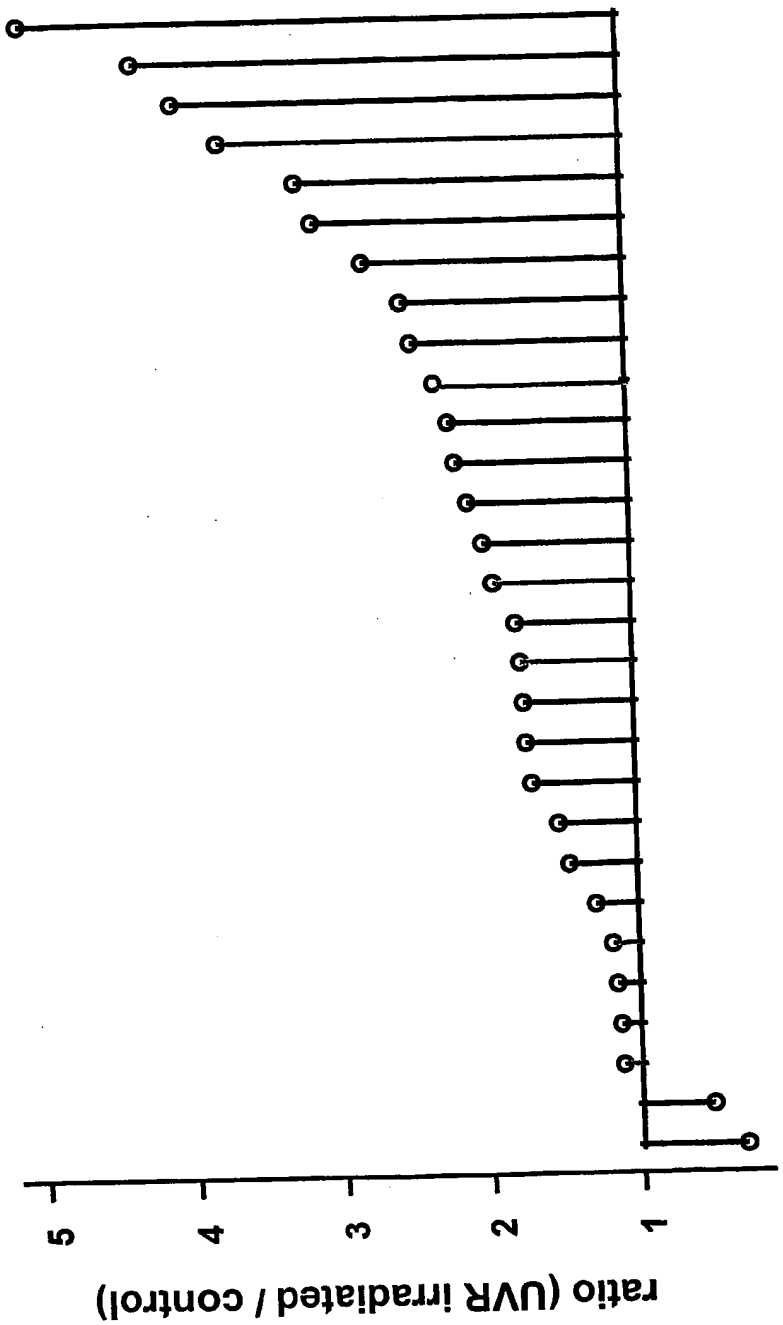
40
35
30
25
20
15
10
5
0
Non-lesional psoriatic skin
SSR-irradiated non-lesional psoriatic skin
Lesional psoriatic skin
Breast
Kidney
Liver
Colon
Placenta
Duodenum
Jejunum



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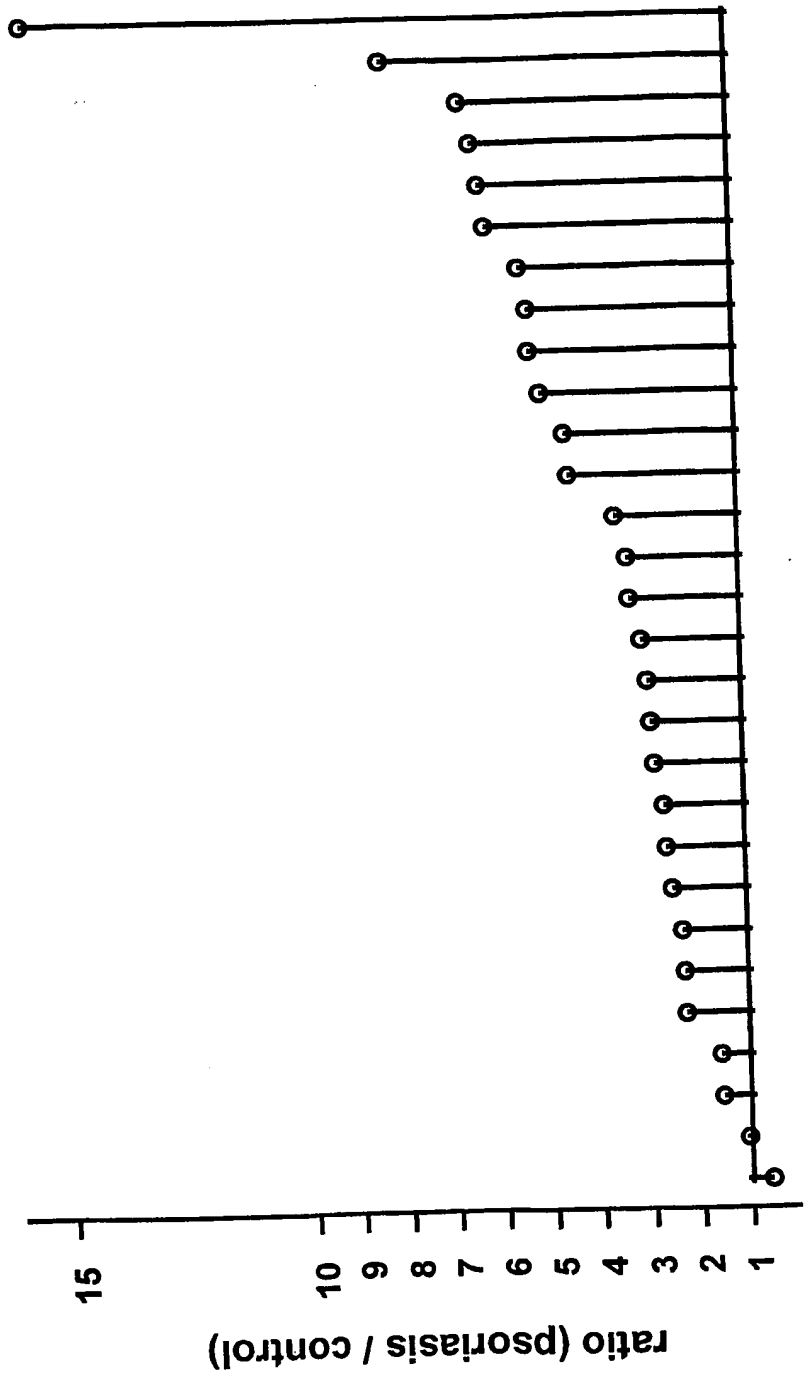

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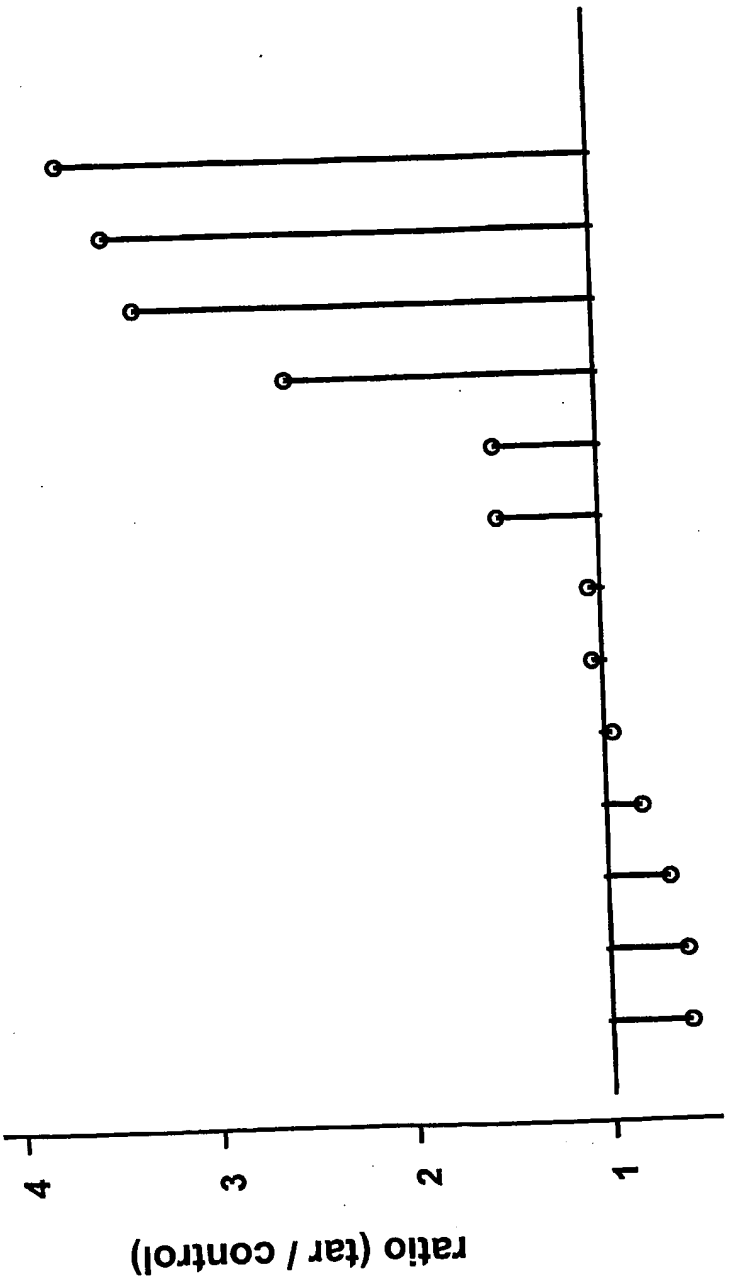


Figure 3A

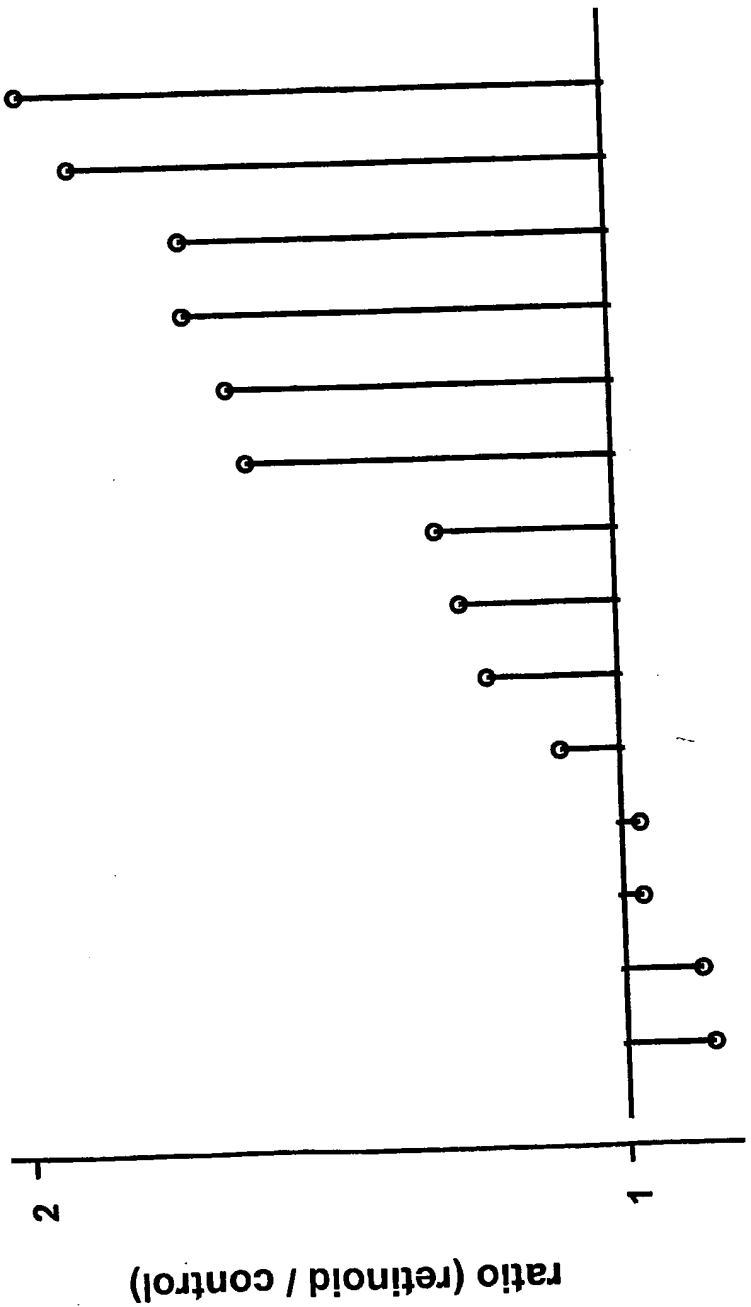


Figure 3B

6/7

Figure 4

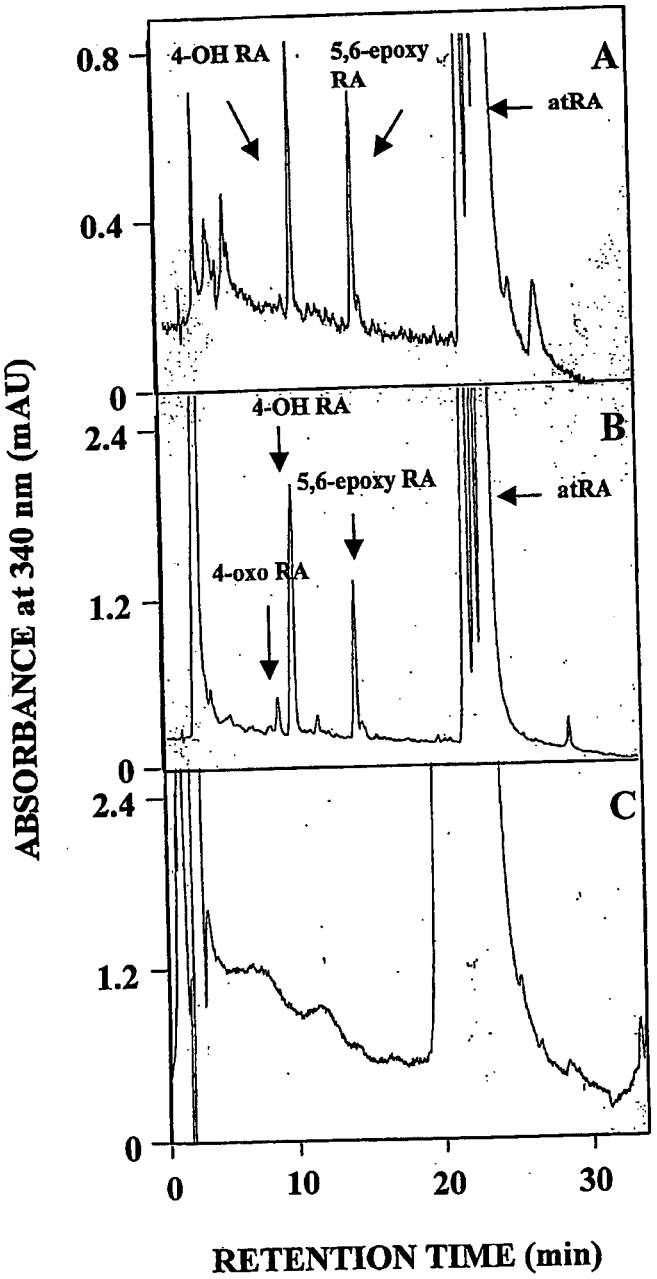
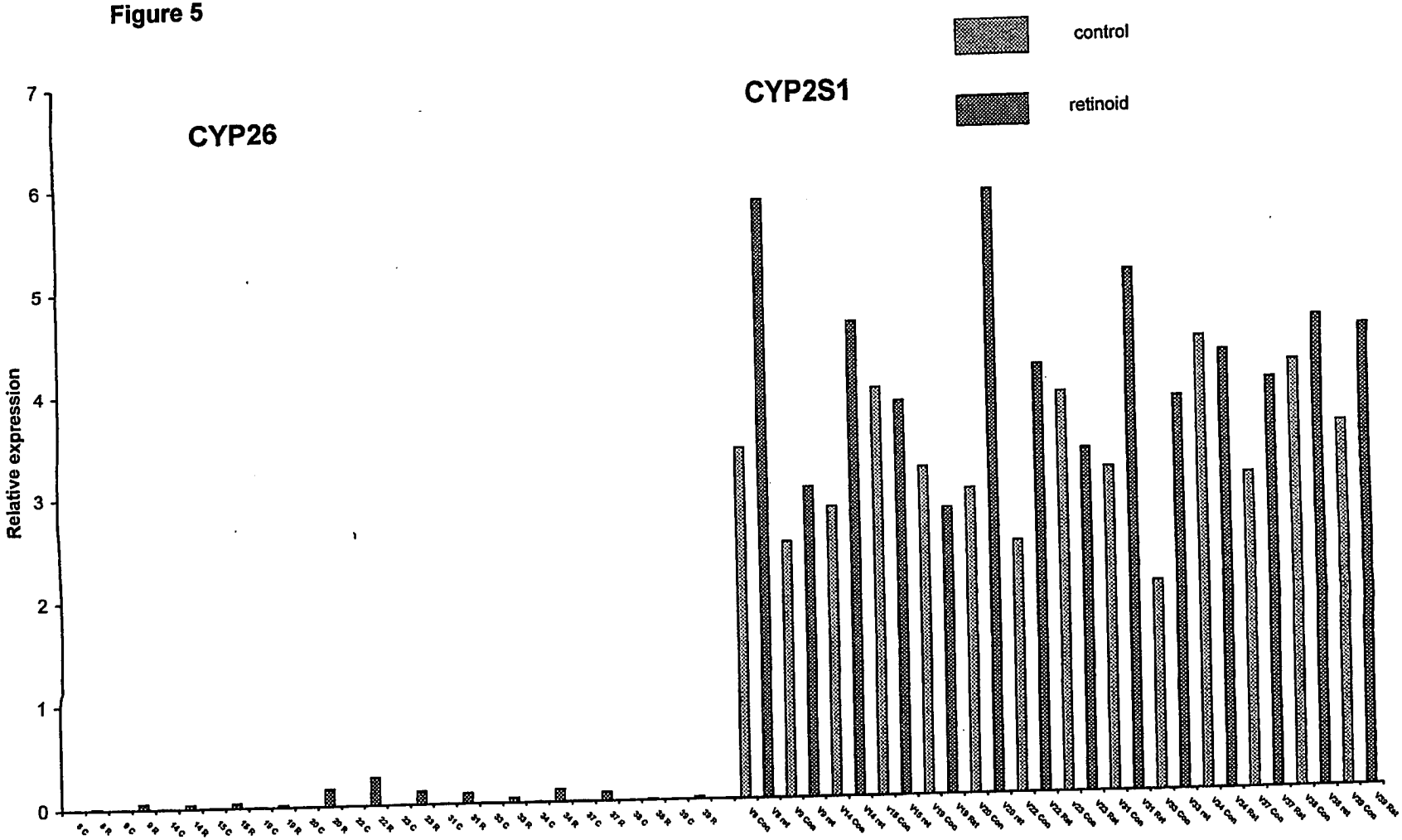

4-OH RA
5,6-epoxy RA
atRA
A
0.8
0.4
0
4-OH RA
5,6-epoxy RA
atRA
4-oxo RA
B
2.4
1.2
0
C
2.4
1.2
0
0
10
20
30
ABSORBANCE at 340 nm (mAU)
RETENTION TIME (min)

Figure 5


control
retinoid
CYP26
CYP2S1
Relative expression
0
1
2
3
4
5
6
7

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